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"EFFECTS OF HYDROSTATIC PRESSURE ON PHOTOSYNTHESIS AND GROWTH OF UNICELLULAR MARINE ALGAE AND DIATOMS."

ABSTRACT

Light-dependent oxygen production and growth of algal cultures have been measured at 25°C at various light intensities and hydrostatic pressures.

A device which maintains a desired concentration of dissolved oxygen during growth and oxygen evolution by photosynthetic organisms is described. The system uses a modified rate measuring oxygen electrode in conjunction with an oxygen concentration monitoring system.

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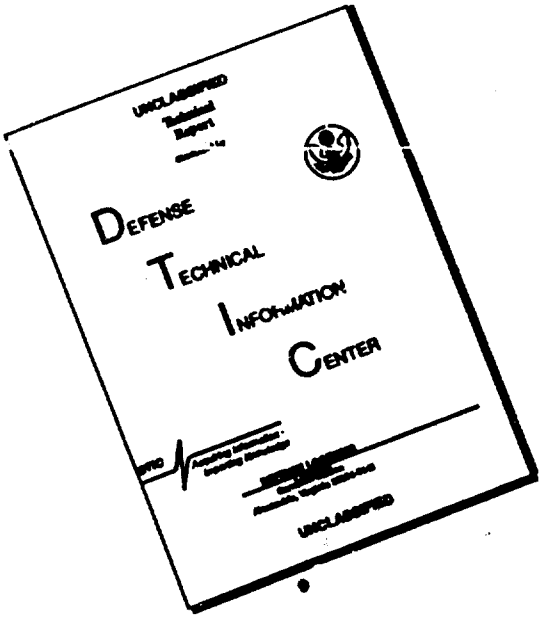

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All work was done at 25°C.

I. Oxygen production by algae at increased hydrostatic pressure.

A. Apparatus.

We are using two types of polarographic oxygen electrodes. The first type, described in the First Annual Progress Report, measures the concentration of dissolved oxygen in the environment without measurably affecting that concentration. This unit depends on an oxygen gradient across a membrane placed between the environment and the electrode per se. Three types of membranes have been used: dimethylsilicone, previously described, Teflon, and polyethylene. We currently use polyethylene.

The second electrode system measures directly the rate of light-dependent oxygen evolution by algal cells. The principle of the unit is basically that described by Chandler and Vidaver (1). A relatively large platinum electrode is placed directly against a membrane filter on which is layered a known number of cells. The cells are supported by dialysis membrane and the whole assembly is immersed in growth medium. The assembled unit is placed in windowed cylinders and may be pressurized to 1000 atmospheres, maximum. Illumination of the cells is external.

B. Results.

The test organism has been a strain of unicellular blue-green alga, Anacystis nidulans for most experiments.

Figure 1 shows the effect of light intensity on the rate of oxygen production. Subsequent studies were done at 750 foot candles. At about this intensity the rate of oxygen production is proportional to the cell concentration over the range 10^7-10^8 cells per membrane filter.

The effects of hydrostatic pressure on these organisms is shown in Figure 2. Within 10 to 20 seconds after the light is turned on a very rapid increase in the rate of oxygen evolution is observed. The actual rate of increase is probably greater than that which can be recorded by our apparatus. The plateau in the curve represents the maximum rate of oxygen production and a constant rate of photosynthesis. Four to five seconds after extinguishing the light the rate of oxygen evolution begins to drop and rapidly reaches a base-line value. Most of the time taken to reach the base-line value is probably instrumental - an artifact of the experimental system.

The rate of oxygen evolution is essentially unaffected by pressure up to 200 atmospheres. A slightly reduced rate occurs at 400 atm. and only approximately 50% of the ambient rate is evident at 600 atmospheres. Figure 3 and Table I summarize these data including experiments made at hydrostatic pressures up to 1000 atm. Marked decreases in the rate of oxygen evolution is observed at higher pressures; no oxygen was evolved at 1000 atmospheres.

The above data are maximum rates of oxygen evolution. Figure 4 shows the relative rate of oxygen evolution by the algae over a 60-minute period. The data are plotted as percent of the values at ambient pressure. At 100 and 200 atm. a new steady-state rate is reached by 60 minutes; above these pressures a new steady-state rate was not reached within the period of the experiment. Presumably the rates fall to zero at pressures above 400 atmospheres (see data on growth below).

We are doing experiments to obtain rapid growth of diatoms in closed culture. To this end application of the method of Berger and Tam (2) is being used. With cultures grown in open systems (but bacteria-free) we have demonstrated that our procedures can measure the rate of oxygen evolution in the light by these organisms.

II. Growth of Anacystis nidulans at increased hydrostatic pressure.

Growth of the blue-green alga at various pressures was determined by placing suspensions of cells in stoppered tubes which were then incubated inside the illuminated pressure vessels. Growth was determined by increase in the density of culture. It was found that depressurization followed by removal of the tubes to determine growth and subsequent replacement and repressurization within 5 or 10 minutes had no effect on the growth rate. Growth was maintained exponentially up to 40 hours in the cylinders. The effects of hydrostatic pressure on the growth of Anacystis nidulans is shown in Figure 5.

III. Growth of algae at increased hydrostatic pressure at constant oxygen concentrations.

Except in open systems, cultures of organisms accumulate oxygen during photosynthetic growth. The concentration of oxygen in the environment affects the rate of growth of the organism and that of photosynthesis itself. Thus, in closed cultures measurement of these parameters is uncontrolled in ordinary laboratory experiments. Open systems are not readily feasible at increased hydrostatic pressures.

We have devised a system which we are currently testing which permits control and maintenance of a desired oxygen concentration in closed pressurized cultures of photosynthesizing algae.

In principle the device utilizes two polarographic electrode systems: a concentration-monitoring electrode whose output is displayed on a strip-chart potentiometric recorder. When oxygen exceeds a preset concentration a switch in the recorder activates the second electrode system whose capacity is large enough to reduce the oxygen to the preset concentration. At this point the second electrode is automatically switched off. An event marker records the period when the electrode is operating. From this information both the rate of oxygen production and the total oxygen produced may be computed. To prevent the large electrode system from becoming poisoned from prolonged exposure to cellular materials, a membrane of dimethylsilicone and electrode gel is used. Oxygen permeability through the membrane is not a limit to the rate of oxygen reduction by the system.

We have encountered a problem of toxicity of our O-rings to Anacystis. Apparently algae are generally sensitive to by-products from rubbers. Coating the Buna-N O-rings with inert materials, or using silicone O-rings appears to be the simplest remedy to this problem. (This suggests a pollution problem of rubber in off-shore waters!)

CONCLUSIONS

1. It is technically feasible to measure the growth rate and rate of oxygen evolution by photosynthetic organisms at increased hydrostatic pressure.

2. It is possible to simulate the dilute marine environment in the laboratory, to introduce light into it, and to control the level of oxygen at any preset level (assuming that the rate of oxygen evolution exceeds that of respiration).

3. It is possible to measure both the rate of change and the absolute quantity of dissolved oxygen in vessels pressurized to 1000 atm. (hydrostatic).

4. The Blue-green alga, Anacystis nidulans grows at pressures exceeding 200 atm. without significant reduction in rate; up to 400 atm. with decreased rate of growth at 25°C, the temperature at which all work was done.

FUTURE DIRECTIONS OF THIS STUDY

No further development of techniques are envisaged during the remainder of the Contract period. We plan to obtain data on the photosynthetic activities of a variety of algae under controlled conditions of hydrostatic pressure, temperature and oxygen concentration. The rate of carbon dioxide incorporation by these organisms will be determined using ^{14}C-labelled bicarbonate.

REFERENCES

1. M. T. Chandler and W. Vidaver Stationary platinum electrode for measurement of O_2 exchange by biological systems under hydrostatic pressure. Rev. Sci. Instr. In press.

2. L. R. Berger and L. Q. Tam 1970. A method to grow obligately aerobic bacteria at increased hydrostatic pressure. Limnol. and Oceanogr. 15, 483-485.

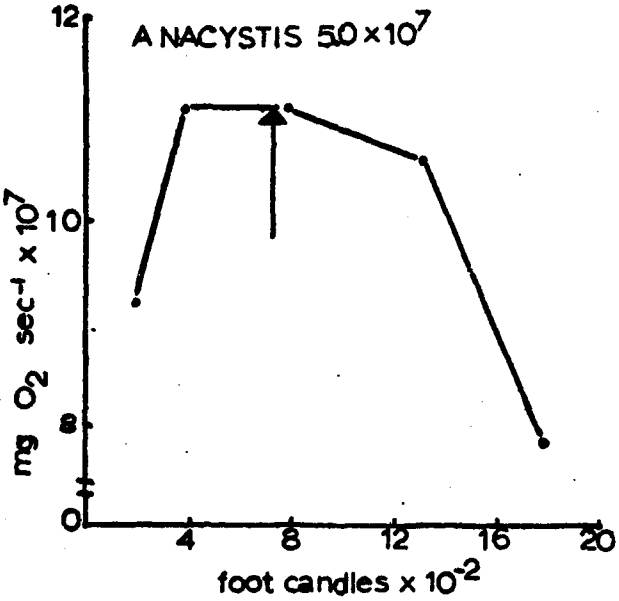

Figure 1. Effect of light intensity on the rate of oxygen evolution by Anacystis nidulans. 5.0 x 10^7 cells were used. 25°C . Ambient pressure.

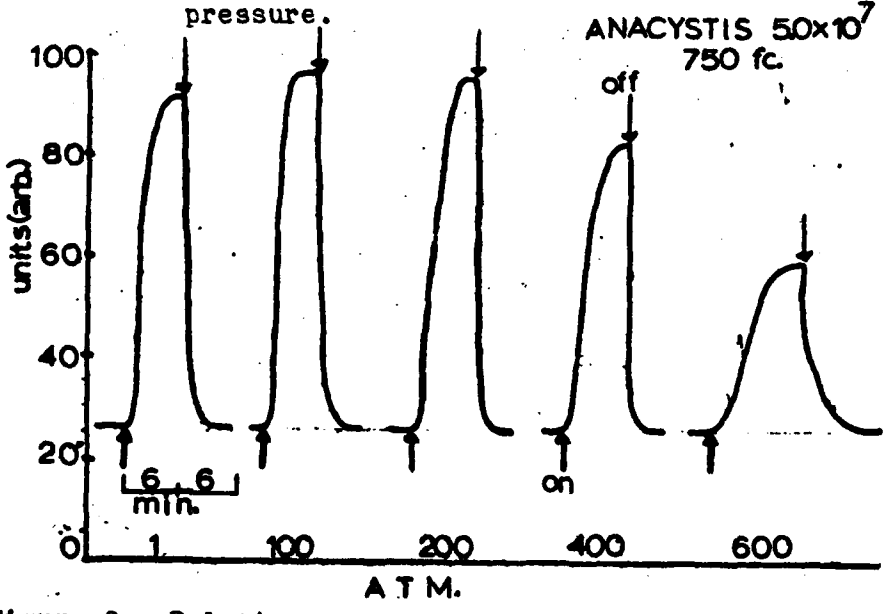

Figure 2. Relative rates of oxygen evolution by A. nidulens at 25°C, 750 ft. candles at various hydrostatic pressures. 5.0 x 10^7 cells were used per determination.

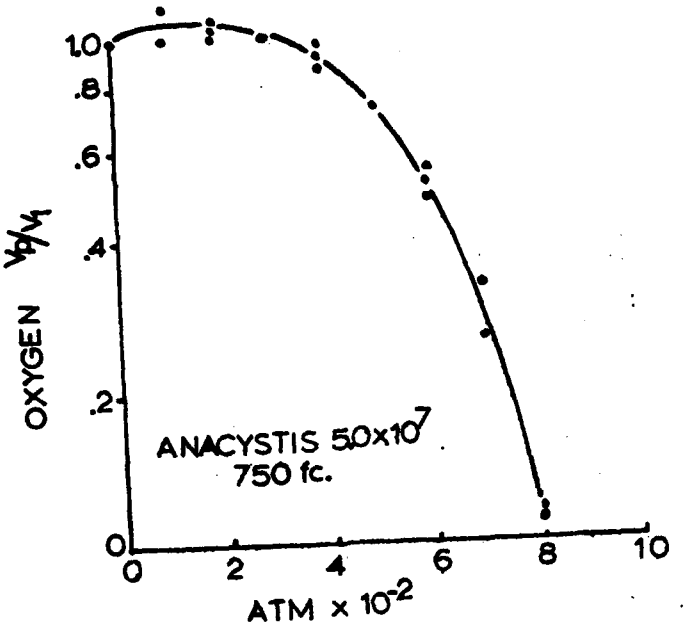

Figure 3. Relative rates of oxygen evolution by A. nidulans vs hydrostatic pressure. See Fig 2. for details. The ordinate is the ratio of observe rate to rate at ambient pressure.

TABLE 1.

ANACYSTIS 1.0×10^8 ml^{-1}

ATM.	rate oxygen evolution volts min^{-1}	
1	0.24	0.18
50	—	0.19
100	—	0.19
300	0.30	0.18
600	0.23	0.14
1000	0.00	—
media 1	0.00	0.00
media 1000	—	0.03

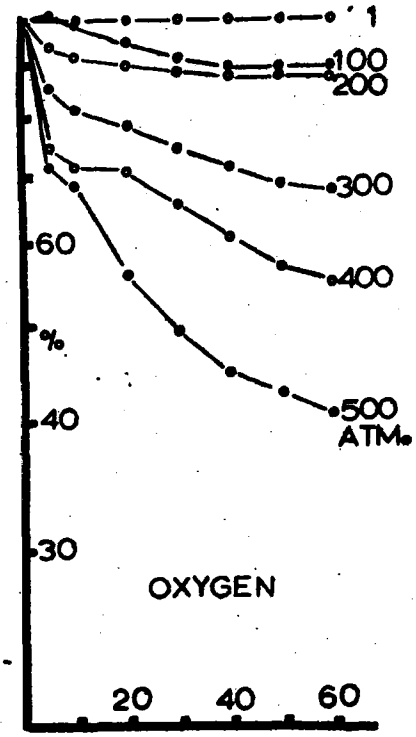

Figure 4.

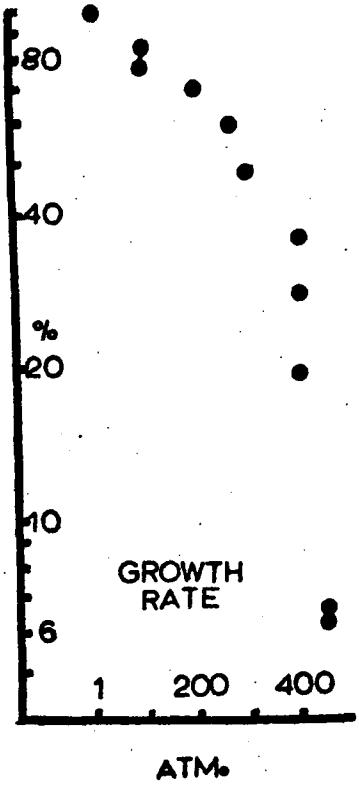

Figure 5.

Figure 4. Relative rate of oxygen evolution by A. nidulans over a 60 minute period. Condions similar to those in Figure 3.

Figure 5. Relative growth rate of A. nidulans in pressurized cultures. Rate was measured during exponential increase of the culture. See text for details.

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